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Extended Summaries International Plant Protection Congress

The following are extended summaries based on papers presented at the International Plant Protection Congress held in The Hague, The Netherlands, 2–7 July 1995. They are entirely the responsibility of the authors and do not necessarily reflect the views of the Editorial Board of Pesticide Science.

Mode of Action of the Novel Rice Blast Fungicide KTU 3616

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KTU 3616, {'Win'®; 1*R*,*S*,3*S*,*R*-2,2-dichloro-*N*-[1-(4-chlorophenyl)ethyl]-1-ethyl-3-methylcyclopropanecarboxamide; proposed common name carpropamid; Fig. 1} provides outstanding protective efficacy, with systemic properties in the control of rice blast (caused by *Pyricularia oryzae* Cavara), and was developed jointly by Bayer AG and Nihon Bayer Agrochem. A single application of the granular formulation to the nursery box a few days before transplanting controls not only leaf blast but also panicle blast. KTU 3616 has a specific mode of action which differs from that of other derivatives of cyclopropane carboxylic acid.

KTU 3616 did not show any remarkable activity against various plant pathogenic fungi and bacteria in culture tests but the colour of the mycelium was changed in many fungi. Spore germination and appressorium formation in *P. oryzae* were not influenced substantially but the pigmentation of appressorium cells was strongly inhibited, even at very low application

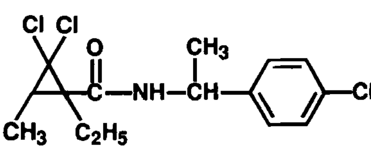

Fig. 1. Structural formula of KTU 3616.

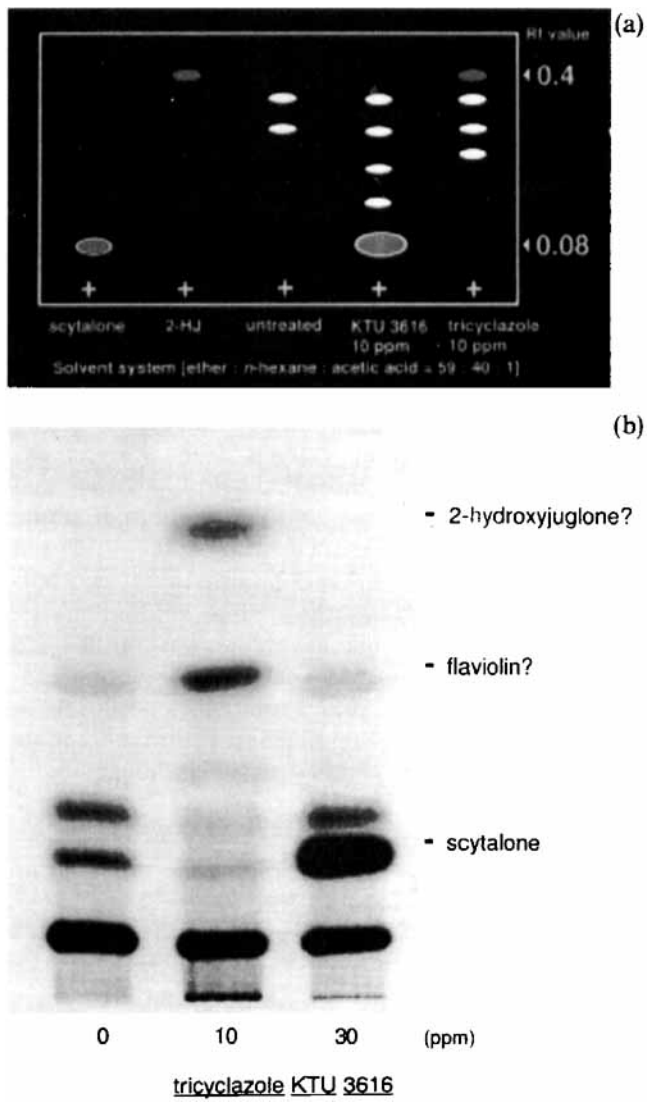

Fig. 2. Inhibition of melanin biosynthesis by KTU 3616. (a) TLC separation of extracts from culture filtrate of *Pyricularia oryzae* treated with KTU 3616 or tricyclazole. (b) Incorporation of [^{14}C]acetate into intermediates of the melanin biosynthesis pathway.

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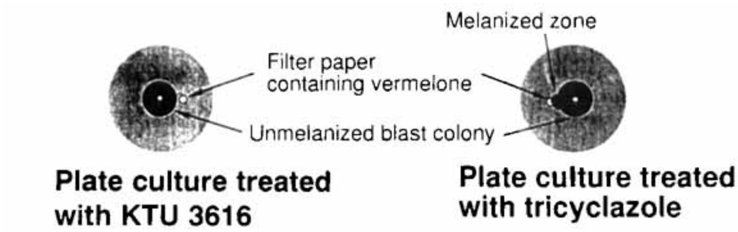
TABLE 1
Inhibition of Vermelone Dehydration by KTU 3616

Intermediate	*Rate (μg)*	*Melanization of mycelium*		
		KTU 3616	*tricyclazole*	*Untreated*
Vermelone	15	–	+	+
1,8-DHN	30	+	+	+



rates, leading to transparent appressoria. These results suggested that KTU 3616 is a melanin biosynthesis inhibitor like tricyclazole, preventing the blast fungus from penetrating into the rice epidermal cell. It was also shown that the compound effectively inhibited appressorial penetration of rice blast into a cellophane membrane.

In order to characterize precisely the target site within the melanin biosynthesis pathway, a liquid culture of *P. oryzae* was treated with KTU 3616. The culture filtrate was adjusted to pH 2 with hydrochloric acid and the pentaketide intermediates of melanin biosynthesis were extracted with ethyl acetate. TLC of the extract resulted in the appearance of a new spot, R_f 0·08 which was absent from extracts from tricyclazole-treated mycelium (Fig. 2(a)). Radio-TLC showed that the same metabolite accumulated in the liquid culture when the fungus was treated with KTU 3616 and sodium [^{14}C]acetate, which is known to be incorporated into the pentaketide pathway (Fig. 2(b)).

The putative pentaketide metabolite was purified and identified by NMR and MS analysis as scytalone [3,4-dihydro-3,6,8-trihydroxy-1(2*H*)naphthalenone].[1] Up to 70 mg of scytalone accumulated per litre of culture broth of KTU 3616-treated rice blast mycelium.

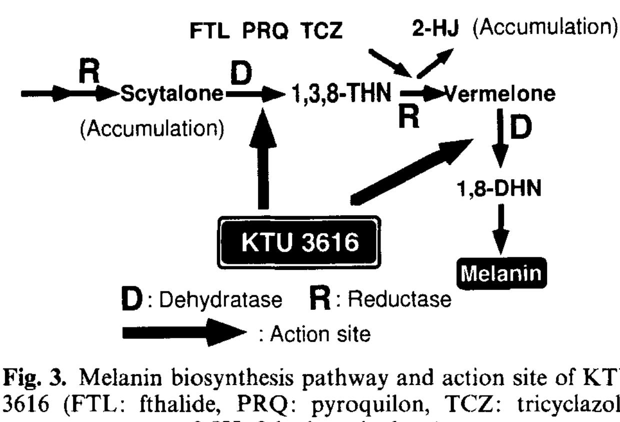


Fig. 3. Melanin biosynthesis pathway and action site of KTU 3616 (FTL: fthalide, PRQ: pyroquilon, TCZ: tricyclazole, 2-HJ: 2-hydroxyjuglone).

Further biological studies were conducted with a crude enzyme extract from *P. oryzae* mycelium using the method reported by Wheeler.[2] Scytalone was metabolized to one or two components which are probably 1,3,8-trihydroxynaphthalene and 2-hydroxyjuglone according to the TLC R_f values. KTU 3616 at <1 mg litre^{-1} strongly inhibited this metabolism indicating that it was an effective inhibitor of scytalone dehydration to 1,3,8-trihydroxynaphthalene.

Reversion experiments with *P. oryzae* colonies on potato dextrose agar plates clearly showed that KTU 3616 inhibited melanization from the vermelone stage, but not from the 1,8-dihydroxynaphthalene stage. Tricyclazole showed no inhibition of the reaction from either intermediate (Table 1). These test results show that KTU 3616 also blocks transformation of vermelone to 1,8-dihydroxynaphthalene, a late step of the melanin biosynthesis pathway.

It is concluded that the primary mode of action of KTU 3616 is the inhibition of melanin biosynthesis by blocking the dehydration of scytalone and vermelone (Fig. 3). This is a novel mechanism different from that of all known melanin biosynthesis inhibitors.

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2-Arylpyrroles: A New Class of Insecticide. Structure, Activity and Mode of Action

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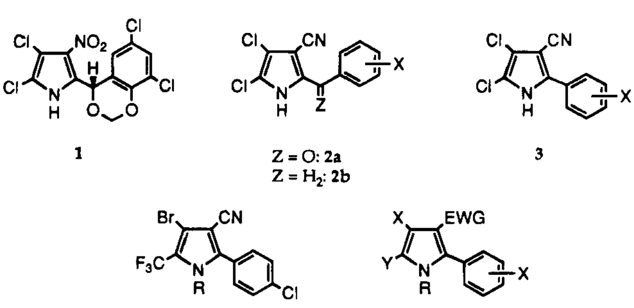
In 1987, the isolation and identification of dioxapyrrolomycin (**1**) from the fermentation of a *Streptomyces fumanus* (Sveshnikova) culture was reported.[1] Dioxapyrrolomycin, a member of the pyrrolomycin family of pyrroles which are highly functionalized with nitro and/or chloro substituents,[2–5] has been shown to possess both antibacterial and antifungal properties.[6,7] Subsequent work in our laboratories established that it gave moderate levels of insect and mite control; however, the levels of insecticidal activity coupled with an observed mouse oral LD_{50} of 14 mg kg^{-1} precluded its development as an agrochemical.

Based on the structure of dioxapyrrolomycin, it was suspected that the observed insecticidal activity could be attributed to the uncoupling of oxidative phosphorylation. This suspicion was subsequently confirmed through mouse-liver mitochondrial assays ($U_{50} = 25$ nM).[8] While the actual mechanism of uncoupling this crucial biological process is poorly understood on the molecular level,[9] the biological manifestations of a molecule which inhibits oxidative phosphorylation are understood to be dependent on two physicochemical parameters: (1) the molecules must be sufficiently lipophilic to move within and across mitochondrial membranes,[10–12] and (2) the molecule must function as both a Brønsted acid and base, thereby disrupting the proton gradient necessary to drive the conversion of ADP to ATP with inorganic phosphate.[10–13] Several pesticides are known to function as uncouplers of oxidative phosphorylation, examples being dinocap,[14] niclosamide,[15] fenazaflor,[16] and carbonyl cyanide phenylhydrazones.[17]

Armed with an understanding of the relationship between lipophilicity (log *P*), acidity (pKa) and uncoupling activity, a synthesis effort was undertaken. Initial structure–activity studies (Fig. 1) addressed the

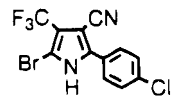
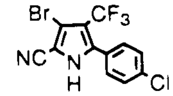
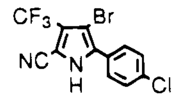
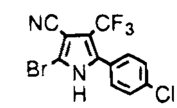


1 Z = O: **2a** Z = H_2: **2b** 3

R = H: **4a** R = $CH_2OC_2H_5$: **4b** 5

Fig. 1. Structures of compounds referred to in the text.

TABLE 1
Insecticidal Efficacy of Various Regioisomers of Compound **4a**[a]

Compound	Mortality at 10 mg liter^{-1} (%)	
	Southern armyworm[b]	*Tobacco budworm*[c]
4a	100	100
	100	0
	0	0
	100	0
	0	0

[a] see Fig. 1.
[b] *Spodoptera eridania* (Cramer), 3rd instar.
[c] *Helicoverpa virescens* (Fabricius), 3rd instar.

importance of the 1,3-dioxane ring and bridge, leading to the preparation of a series of 2-aroyl- (**2a**) and 2-benzylpyrroles (**2b**). Additional studies investigated the effect of eliminating the keto and methylene spacer, resulting in a series of 2-arylpyrroles. While these structural simplifications resulted in compounds possessing moderate-to-good levels of insecticidal activity, a substantial increase in efficacy was achieved by the replacement of the 5-halogen by trifluoromethyl in the 2-aryl-3-cyano-4,5-dihalopyrrole series (**3**), thus leading to a series of compounds exemplified by 4-bromo-2-(4-chlorophenyl)-5-trifluoromethylpyrrole-3-carbonitrile (**4a**).[18]

The insecticidal activity of the various regiosomers of the trifluoromethyl pyrroles bearing a 2-(*p*-chlorophenyl) substituent is outlined in Table 1. As might be expected, resonance and inductive effects of the functional groups directly affect the pKa of the pyrrole -NH. Therefore, the relative positions of these substituents on the pyrrole ring have a marked effect on the level of insecticidal activity.

Since plants as well as insects utilize ATP as an energy source, problems with phytotoxicity were observed and necessitated efforts toward the development of an insect-selective pro-drug tactic. This strategy would protect the plant while releasing the toxicant selectively to the target pest *via* an insect-selective metabolic pathway. We found that the *N*-ethoxymethyl group provided the desired effect. For example, the

pyrrole **4a** was phytotoxic and had an observed U_{50} = 2.4 nM in a mouse-liver mitochondrial assay. The corresponding protected pyrrole **4b** was not phytotoxic, had the same spectrum of activity, and had an $U_{50} > 1000$ nM in the mouse-liver mitochondrial assay. Moreover, in a potato leaf-dip assay, it was found that when Colorado potato beetle [*Leptinotarsa decemlineata* (Say)] adults were exposed to the microsomal monooxygenase inhibitor piperonyl butoxide, they were significantly less sensitive to **4b**. These experiments clearly demonstrate that the actual toxicant moiety is the NH-pyrrole and its formation must be activated by the herbivore *via* enzyme-mediated oxidative removal of the *N*-ethoxymethyl group; foliage-chewing insects (Lepidoptera, Coleoptera) are known to readily oxidize xenobiotics.[19]

From the knowledge of the balance required between log *P* and pKa, structure–activity relationship studies have established that the best activity observed for the 2-arylpyrrole series exemplified by **5** requires X = Br or Cl, $Y = CF_3$, and an EWG (electron-withdrawing group) chosen from CN, NO_2 or $S(O)_{(0-2)}CF_3$. Substitution on the 2-aryl ring system is critical, both in terms of inductive effect and position. The substituent must be capable of some electron withdrawal (e.g. Cl, Br, CF_3), with the optimal substitution at the 4-position. Analogs with 3,4-disubstitution on the phenyl ring also possess good levels of insecticidal activity.

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